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BIOLOGICAL TRACE ELEMENT MEASUREMENTS  
USING SYNCHROTRON RADIATION

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ABSTRACT

The feasibility of performing x-ray fluorescence trace element determinations at concentrations substantially below the ppm level for biological materials is demonstrated. Conditions for achieving optimum sensitivity were ascertained. Results achieved for five standard reference materials were, in most cases, in excellent agreement with listed values.

Minimum detectable limits of 20 ppm were measured for most elements.

INTRODUCTION

The availability of intense, linearly polarized synchrotron radiation has led to significant interest in applications involving x-ray fluorescence analysis (1-7). The high intensity of these beams permits the use of tunable crystal monochromators which provide variable energy narrow bandwidth excitation radiation. The linear polarization inherent in the incident beam makes possible the reduction of scattered background with corresponding improvement in analytical sensitivity for trace elements.

Although these basic properties of x-ray fluorescence using monochromatic synchrotron radiation have been demonstrated, very little quantitative trace element data have been obtained and verified by comparison with artificial standard samples. In this paper we report the quantitative analysis of several Standard Reference Materials with emphasis on sample types of interest for biological analysis. These results can be used as indicators of the minimum

detectable limits, and analytical accuracy which can be achieved for biological specimens.

#### EXPERIMENTAL PROCEDURES

The procedures used in the measurements were identical to those reported in Ref. 8. The radiation obtained from the LBL/Exxon 54-pole wiggler (9) beam line at the Stanford Synchrotron Radiation Laboratory (SSRL) was focused using an x-ray mirror. A silicon <220> double crystal monochromator selected a narrow portion ( $\Delta E/E \sim 10^{-4}$ ) of the continuous radiation spectrum with maximum energy of 20 keV. The experimental geometry is shown in Figure 1. Although the area of the incident beam was set to a 3 x 3 mm cross section using variable horizontal and vertical slits, the intense region of the beam spot was only 1 mm high. The specimens were placed at an angle of  $45^\circ$  to the incident radiation and were continuously scanned across a 4 x 17 mm area during the data acquisition in order to average out sample or beam inhomogeneities. A 5 mm thick,  $80 \text{ mm}^2$  Si(Li) detector was carefully aligned so that it was at the beam height and at  $90^\circ$  to the path of the incidence radiation. This position minimizes scattered radiation into the detector since the beam from the wiggler is about 94% linearly polarized. A pair of 6 mm diameter collimators shielded the periphery region of the detector. An effective solid angle  $\Omega/4\pi = 3.5 \times 10^{-4}$  was viewed by the detector. Maximum incident fluxes of  $3 \times 10^{12}$  and  $1.6 \times 10^{13}$  photons/sec/mm<sup>2</sup> were obtained at 18 and 10 keV, respectively, when the synchrotron was operated at 3.0 GeV and 100 mA current. A pair of ionization chambers was used to monitor the beam flux a) incident to and b) transmitted by the specimen. The input count rate seen by the semiconductor detection system was maintained at approximately 10,000 counts/sec by detuning the second monochromator crystal. Corrections for detector system dead time were made using an empirical curve relating detector throughput to input rate.

## Calibration

Two multielement thin standards whose x-ray energies spanned the fluorescence range of interest were used to calibrate the system. The first standard contained Ca, Ti, Mn, Cu, and Ge and the second contained V, Fe, Ni, Zn, Rb, Sr, and Pb. The standards were prepared by aspirating dilute solutions containing the elements into a large chamber connected to the intake line of a dichotomous aerosol sampler. Particles less than 2.5 micron diameter were collected onto stretched polytetrafluoroethylene (PTFE) filters, 37 mm in diameter. The deposits were made thin enough that x-ray absorption due to mass loading was minimal. Relative elemental concentrations were designed to yield similar response for all elements on the standard when excited by monochromatic radiation.

Precise mass loadings of the individual elements on the two standards were determined at our laboratory using a calibrated x-ray spectrometer. Absolute calibration of this spectrometer for a single element, Cu, was achieved using several evaporated gravimetric thin film standards prepared at LBL. Calibration for nearly all other elements was realized from multielement standard solution deposits collected from the fine stage of the dichotomous sampler in a manner similar to that described above.

Figure 2 illustrates spectra obtained at SSRL for our standards using 18 keV excitation radiation. Individual element concentration values in  $\mu\text{g}/\text{cm}^2$  are listed above each element. It should be noted that the two spectra shown were collected over 200 sec intervals using radiation fluxes which differed by a factor of 1.62. The two standards were run before and after each of the five standard reference materials which were analyzed. Absolute response for each element using 18 keV excitation radiation is shown in Fig. 3. Very high precision for the synchrotron measurement was achieved

using the two standards and is reflected by an overall  $2\sigma$  deviation of 1.9% for the 12 elements.

### Sample Preparation

For our experiments five trace element Standard Reference Materials, SRMs, were utilized. Included were three from the National Bureau of Standards—SRM 1549 Non-Fat Milk Powder, SRM 1567 Wheat Flour, SRM 1568 Rice Flour, and two from the National Institute for Environmental Studies in Japan—NIES-4 Freeze Dried Serum, and NIES-5 Hair. These standards represent a typical biological specimen consisting of trace elements uniformly distributed in a hydrocarbon matrix. Weighed standard reference materials were compressed into 2.54 cm disks at a pressure of  $700 \text{ kg/cm}^2$ . No binder material was used. All reference materials were prepared in triplicate. Disk mass thicknesses were in the range of 40 to  $60 \text{ mg/cm}^2$ .

### Matrix Corrections

The specimens were assumed to have a mass absorption coefficient curve of the same slope as that of a typical hydrocarbon matrix with a hydrogen/carbon atomic weight ratio of 2. One point on the curve, at 10 keV, was determined experimentally using transmission data for 10 keV excitation radiation. For each SRM, transmission measurements were made on only one disk, and the results determined were applied to all three disks since they were of very similar mass thicknesses. These measurements were achieved using 100 sec count intervals and using ionization chamber  $I/I_0$  ratio values, with and without the specimen, to calculate the value of  $\mu_m$  for the specimen at 10 keV.

### Minimum Detectable Limits

To establish minimum detectable limit (MDL) curves for hydrocarbon matrices, excitation radiation for energies varying from 6.6 to 20 keV was used to acquire spectra for a blank microcrystalline cellulose disk of mass thickness  $40 \text{ mg/cm}^2$ . Additional spectra were acquired for one of the multi-element standards at each of the excitation radiation energies utilized. MDL curves established for four elements Mn, Zn, Se, and Rb are shown in Fig. 4. These curves are for 300 sec count intervals. The MDLs include matrix absorption corrections. Comparable MDLs could have been realized for thinner specimens since in most cases the double crystal monochromator was detuned to reduce the incident photon flux by as much as a factor of 5 in order to maintain a  $10 \text{ K counts/sec}$  input rate to our detector system.

MDLs approximately 20 ppb were measured for most of the elements that we determined. However, since only one energy of excitation radiation is normally utilized for analysis, MDLs actually realized for some elements can increase by as much as a factor of 5. It should be noted that the curves in Fig. 4 illustrate that the optimum MDL for any given element was achieved when the excitation radiation is of energy substantially above the K shell absorption edge energy.

In some cases, sensitivity for a given element can be substantially enhanced relative to other elements present in the specimen by optimization of the excitation radiation energy. The ability to precisely tune the incident beam energy over a wide energy range enables one to fully exploit monochromatic excitation. In one of our earlier experiments (10), we were able to measure the concentration of As at the  $2 \text{ ng/cm}^2$  level in an atmospheric aerosol specimen which contained  $460 \text{ ng/cm}^2$  Pb by selecting an excitation radiation of energy 12.900 keV, which is below the Pb L absorption edge yet above the As K absorption edge.



## RESULTS

Except for NBS SRM 1549 Non-Fat Milk Powder, our trace element determinations for the SRMs were carried out using an excitation radiation energy of 18.00 keV. For this former SRM, excitation energies of 10.00 keV and 18.00 keV were employed. Figure 5 clearly illustrates that the sensitivities realized for the determination of the elements Mn and Ni were substantially enhanced by using 10.00 keV photons. Figure 6 is a spectrum acquired for NBS SRM 1568 Rice Flour. The elements Ni, As, Se, and Sr are all present at concentration levels less than 500 ppb and significant x-ray line intensities were realized. Counting intervals of 300 sec were utilized for our determinations. The mean results for the triplicate determinations for the three NBS and the two NIES SRMs are listed in Tables 1 and 2. The errors shown for the XRF determinations are  $2\sigma$  values for calibration and counting errors. In nearly all cases, excellent results were achieved. Also shown are results ascertained for some elements not reported by NBS, Sr for all three SRMs and Ni for SRM 1549.

A spectrum illustrative of an application which exploits the intensity of the excitation is shown in Fig. 7. These data were obtained from a 2  $\mu$ l drop-let of whole blood (air dried). The concentrations listed are in  $\mu$ g/g. Since the air drying concentrated the whole blood by a factor of 5, the concentrations of the listed elements in the original whole blood were a factor of 5 lower. A beam spot size of 2 x 2 mm was utilized for 17 keV excitation radiation and counts were acquired over a 400 sec interval. The Compton scattered radiation intensity served as a measure of sample mass. If the specimens are relatively thin--on the order of several  $\text{mg}/\text{cm}^2$ --matrix absorption effects are essentially negligible for all the elements that we determined except for K and Ca. The results shown in Fig. 7 could easily have been achieved using a much smaller specimen or a reduced beam spot size. This opens up the

possibility of performing analysis of extremely small samples with sensitivities below the ppm level.

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FIGURE CAPTIONS

- Fig. 1. Schematic of x-ray fluorescence analysis technique.
- Fig. 2. Spectra acquired for the two multielement thin standards using 18 keV excitation radiation.
- Fig. 3. Calibration curve established for 18 keV excitation radiation.
- Fig. 4. Minimum detectable limit curves established for microcrystalline cellulose disk of mass thickness  $40 \text{ mg/cm}^2$ .
- Fig. 5. Spectra obtained for NBS SRM 1549 Non-Fat Milk Powder using 10 and 18 keV excitation radiation.
- Fig. 6. Spectrum acquired for NBS SRM 1568 Rice Flour using 18 keV excitation radiation.
- Fig. 7. Spectrum of  $2 \mu\text{l}$  droplet of whole blood air dried.

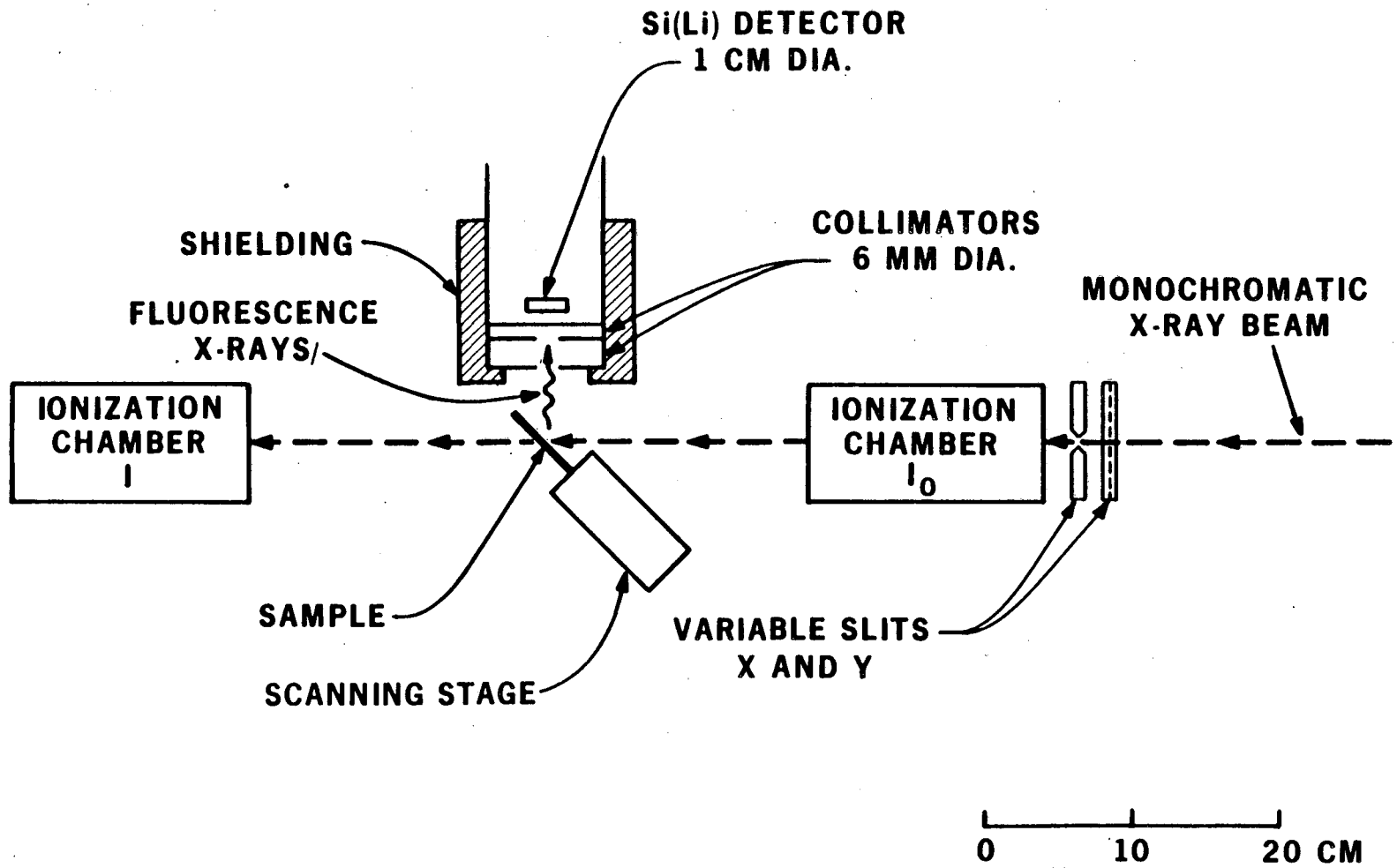
Table 1. Results Determined for Three NBS Standard Reference Materials

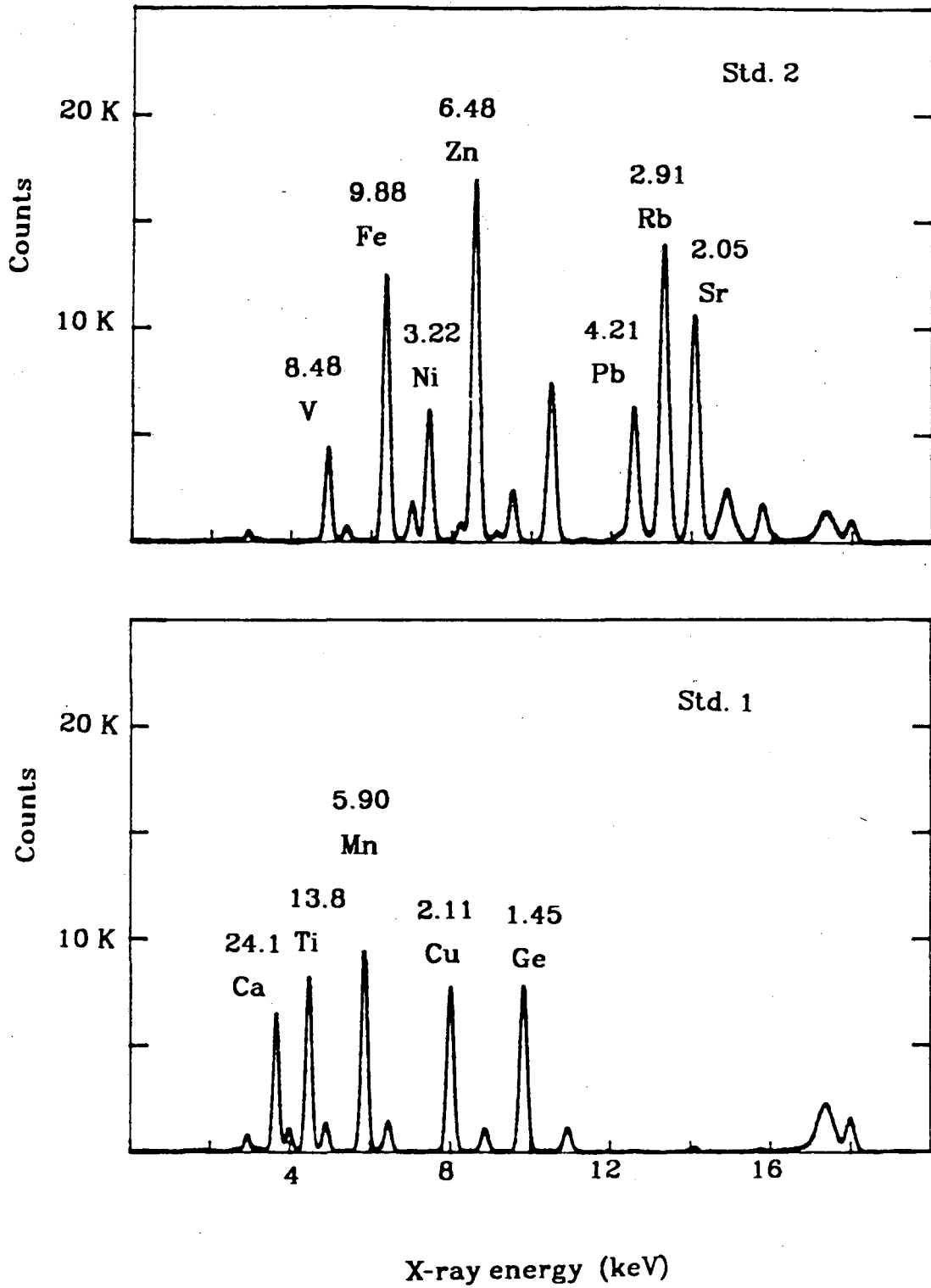
Mass Thickness of Disks Wt. of Area Scanned Recommended Sample Wt.	Non-Fat Milk Powder SRM 1549		Wheat Flour SRM 1567		Rice Flour SRM 1568	
	51 mg/cm <sup>2</sup> 37 mg 500 mg		60 mg/cm <sup>2</sup> 43 mg 400 mg		60 mg/cm <sup>2</sup> 43 mg 400 mg	
Element	NBS	XRF	NBS	XRF	NBS	XRF
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
K	16900 ± 300	17800 ± 2000	1360 ± 40	1220 ± 130	1120 ± 20	1360 ± 160
Ca	13000 ± 500	12000 ± 800	190 ± 10	174 ± 10	140 ± 20	158 ± 14
Cr	0.0026 ± 0.0007	< 0.6	-	< 0.3	-	< 0.4
Mn	0.26 ± 0.06	0.33 ± 0.12	8.5 ± 0.5	8.2 ± 1.8	20.1 ± 0.4	22.1 ± 2.8
Fe	(2.1)	2.30 ± 0.16	18.3 ± 1.0	17.1 ± 4.8	8.7 ± 0.6	9.1 ± 1.2
Ni	-	0.24 ± 0.06	(0.18)	0.11 ± 0.06	(0.16)	0.18 ± 0.06
Cu	0.7 ± 0.1	0.65 ± 0.04	2.0 ± 0.3	1.88 ± 0.12	2.2 ± 0.3	2.21 ± 0.22
Zn	46.1 ± 2.2	46.9 ± 0.9	10.6 ± 1.0	10.3 ± 0.4	19.4 ± 1.0	21.9 ± 1.8
As	(0.0019)	< 0.05	(0.006)	< 0.03	0.41 ± 0.05	0.42 ± 0.09
Se	0.11 ± 0.01	0.09 ± 0.04	1.1 ± 0.2	0.92 ± 0.06	0.4 ± 0.1	0.38 ± 0.04
Br	(12)	12.1 ± 0.2	(9)	8.5 ± 1.4	(1)	1.19 ± 0.17
Rb	(11)	13.1 ± 0.2	(1)	0.94 ± 0.06	(7)	8.4 ± 0.9
Sr	-	3.69 ± 0.10	-	0.82 ± 0.04	-	0.19 ± 0.04
Hg	0.0003 ± 0.0002	< 0.1	0.001 ± 0.0008	< 0.06	0.0060 ± 0.0007	< 0.08
Pb	0.019 ± 0.003	< 0.1	0.020 ± 0.010	< 0.1	0.045 ± 0.010	0.10 ± 0.09

Table 2. Results Determined for Two NIES Standard Reference Materials

Mass Thickness of Disks Wt. of Area Scanned Recommended Sample Wt.	Freeze-Dried Serum NIES-4		Hair NIES-5	
	40 mg/cm <sup>2</sup> 29 mg -		40 mg/cm <sup>2</sup> 29 mg 200 mg	
Element	NIES	XRF	NIES	XRF
	µg/g	µg/g	µg/g	µg/g
K	2140 ± 140	2360 ± 260	34 ± 3	56 ± 16
Ca	970 ± 120	970 ± 130	728 ± 30	904 ± 55
Ti	-	-	(22)	29 ± 4
Cr	0.04 ± 0.03	< 0.5	1.4 ± 0.2	1.8 ± 0.7
Mn	-	< 0.4	5.2 ± 0.3	5.6 ± 0.3
Fe	13.3 ± 0.9	15.2 ± 3.4	225 ± 9	241 ± 17
Ni	-	< 0.2	1.8 ± 0.1	1.8 ± 0.3
Cu	12.7 ± 1.3	12.8 ± 1.0	16.3 ± 1.2	16.9 ± 1.3
Zn	10.8 ± 1.6	11.5 ± 0.9	169 ± 10	178 ± 16
Ga	-	< 0.07	-	0.3 ± 0.2
As	-	< 0.05	-	< 0.2
Se	(1.6)	1.47 ± 0.07	(1.4)	1.6 ± 0.2
Br	78 ± 47	97 ± 8	(90)	102 ± 13
Rb	2.8 ± 1.0	2.42 ± 0.25	(0.19)	0.3 ± 0.2
Sr	(0.6)	0.62 ± 0.06	2.3 ± 0.2	2.4 ± 0.3
Ba	-	-	(3.2)	-
Hg	-	< 0.1	4.4 ± 0.4	4.8 ± 0.5
Pb	-	0.20 ± 0.14	(6.0)	6.4 ± 0.8

Figure 1.

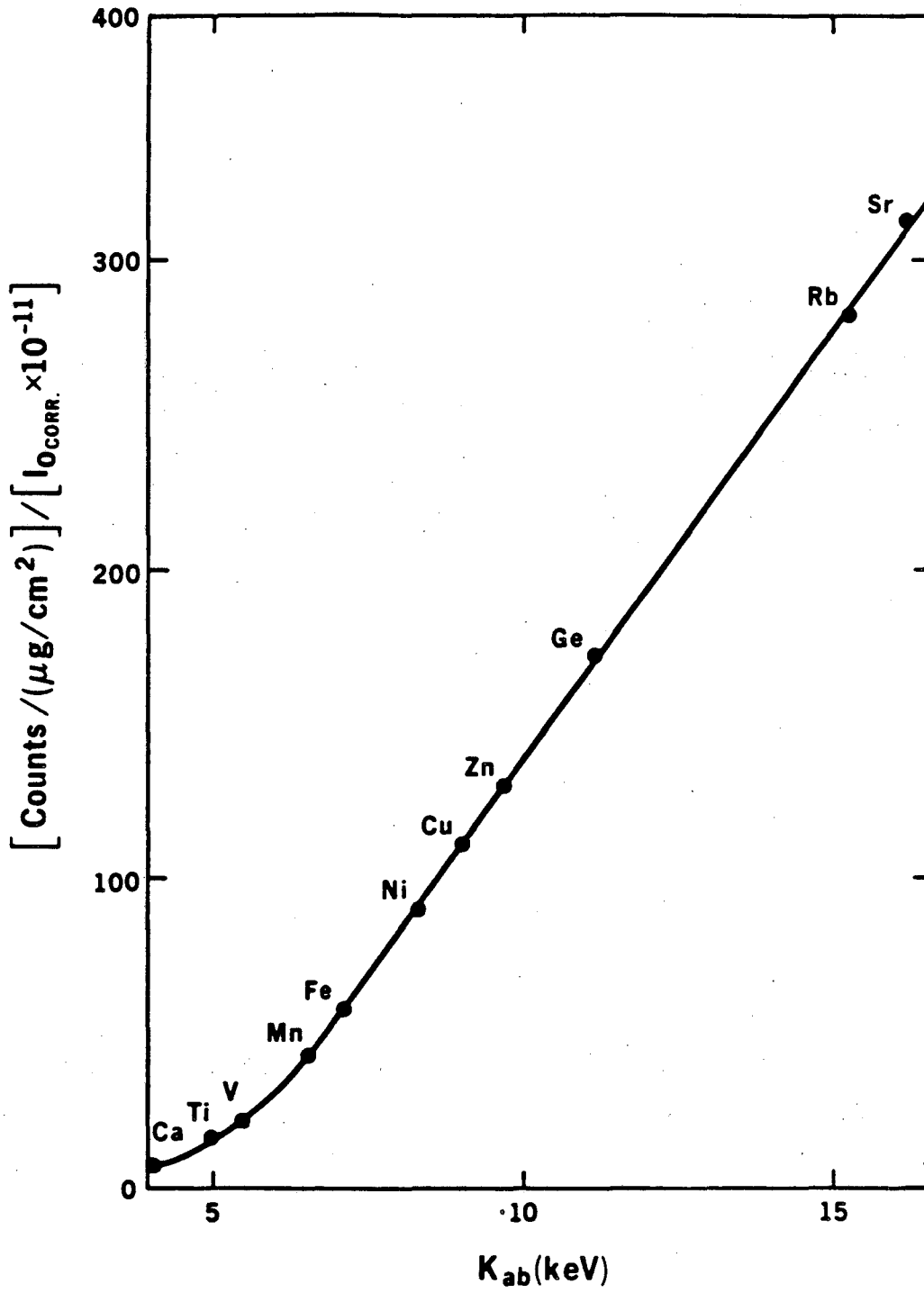




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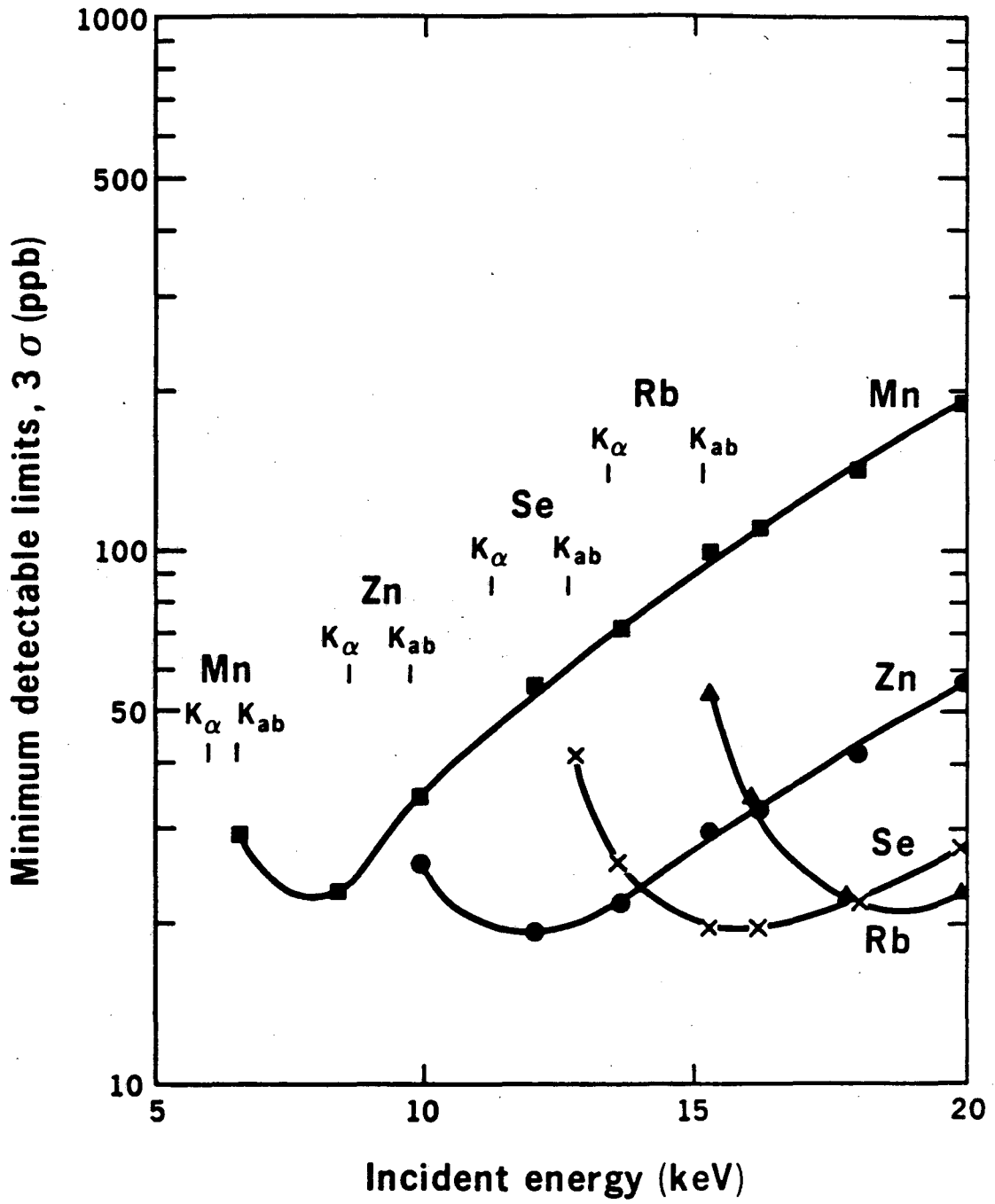
Figure 2.





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Figure 3.



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Figure 4.

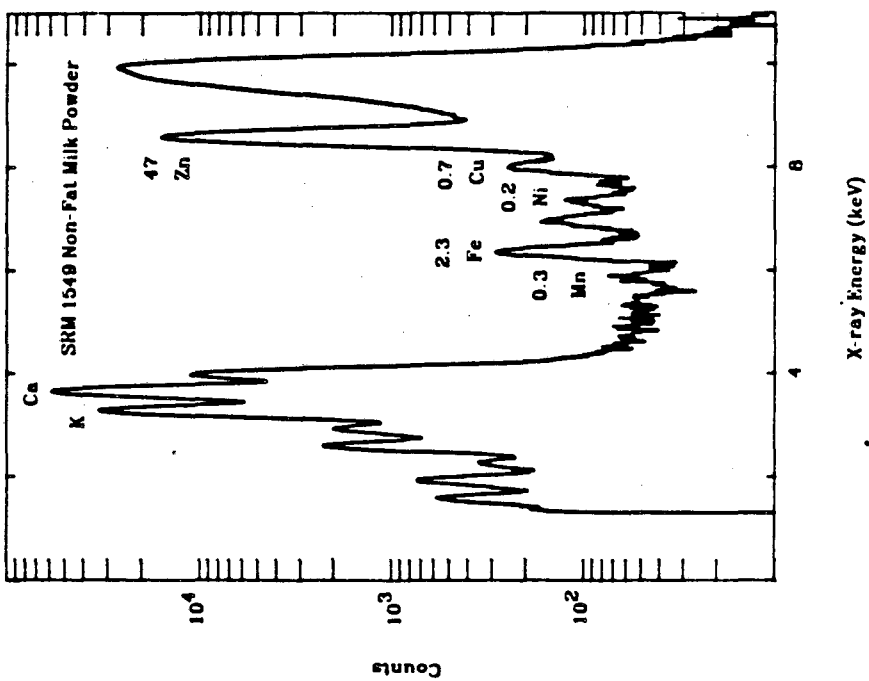
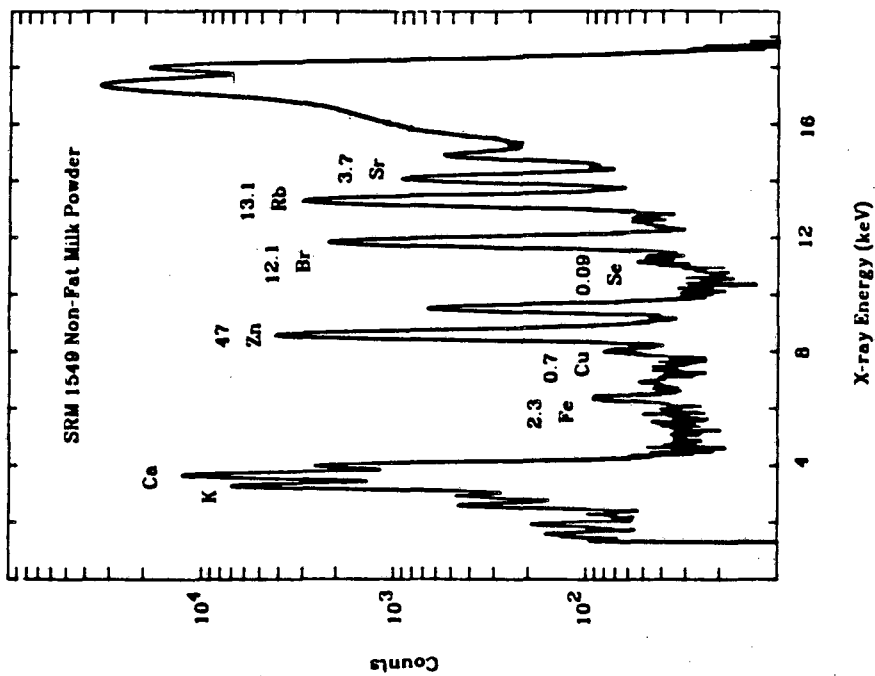
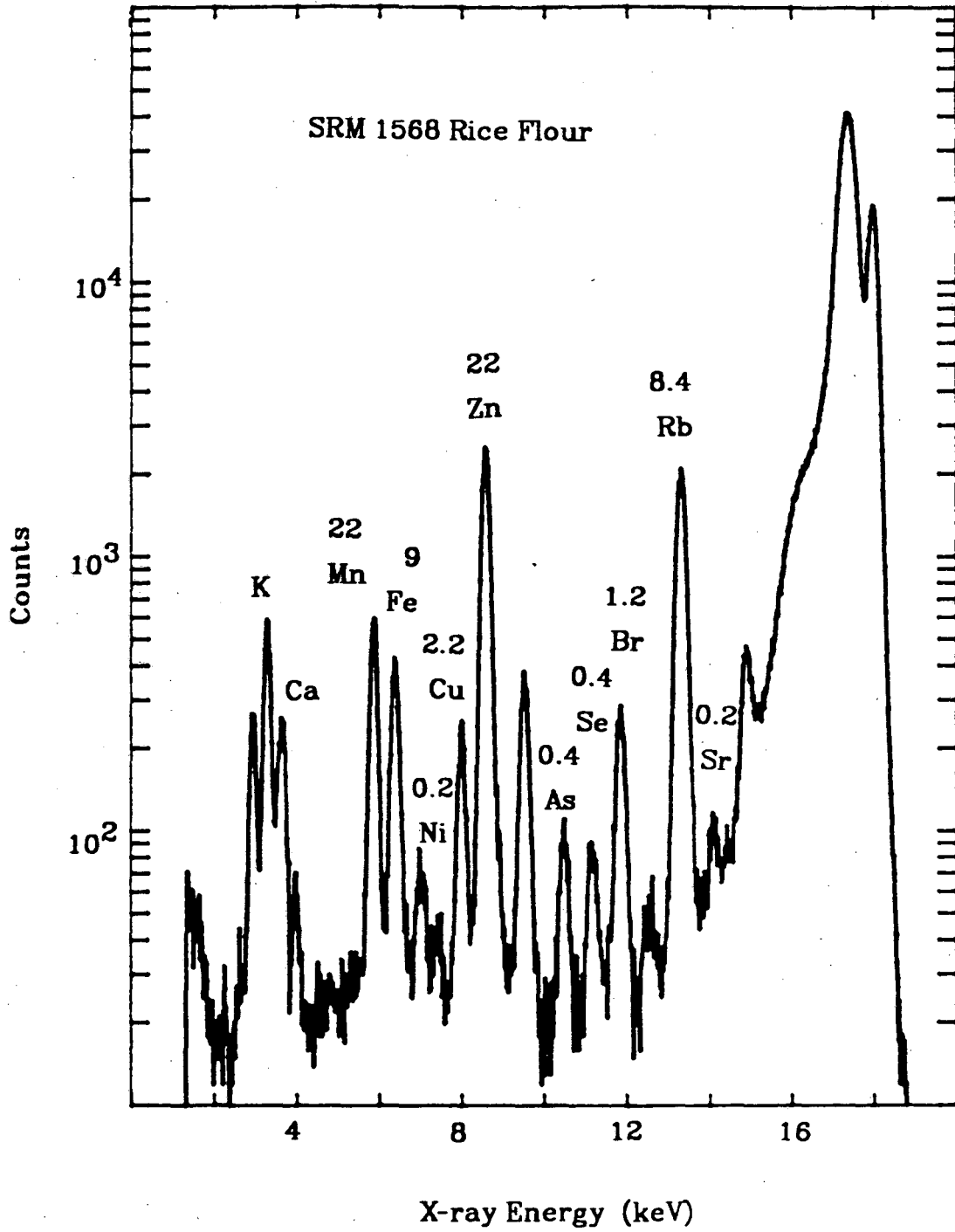


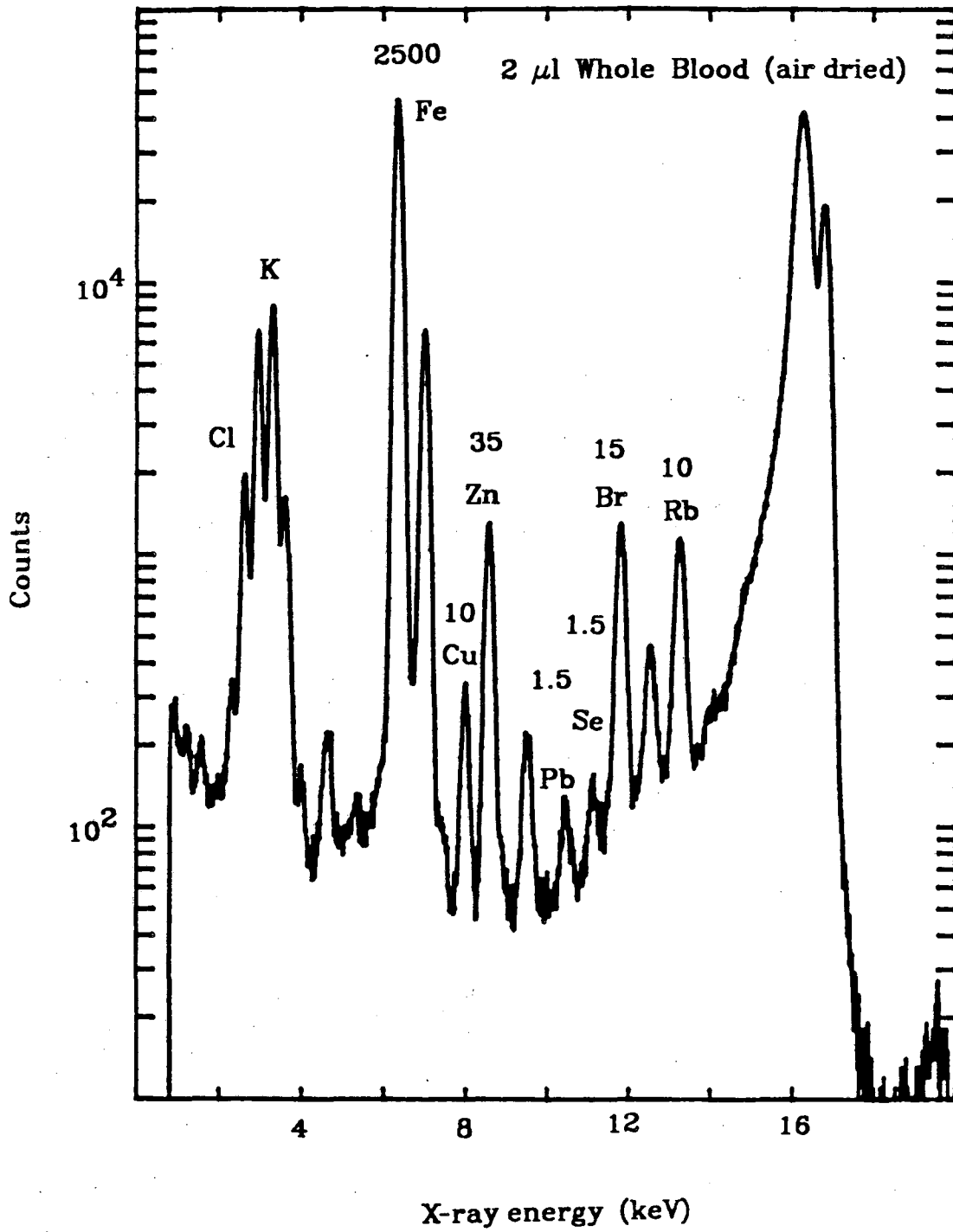
Figure 5.

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Figure 6.



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Figure 7.

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